The Distribution of Hepatitis C Virus Genotypes of Patients with Chronic Hepatitis C Infection in the Eskisehir Region of Turkey

Kronik Hepatit C Enfeksiyonlu Hastalarda Hepatit C Virüs Genotiplerinin Eskişehir Bölgesindeki Dağılımı

HCV Genotipleme

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Özet

Amaç: Hepatit C, karaciğeri etkileyen bulaşıcı ve ciddi bir hastalıktır. HCV'ye bağlı enfeksiyonlarda farklı genotiplerin, hastalık tablosu ve tedaviye cevapta farklılıklar ortaya çıkardığı bu nedenle genotip belirlenmesinin hastaların klinik takip ve tedavisinde önemli olduğu bilinmektedir. Bu çalışma kronik hepatit C hastalarının tedaviye yanıt durumlarında fikir vermesi açısından HCV genotip dağılımının belirlenmesi amacıyla yapılmıştır. Gereç ve Yöntem: Araştırmada 2009-2014 yılları arasında, ESOGÜ Tıp Fakültesi Hastanesinde farklı kliniklerde izlenen 203 hastanın Anti-HCV, HCV RNA viral yükü ve HCV genotip dağılımı değerlenmiştir. Anti-HCV, mikropartikül ELISA kitiyle otomatize sistemde (Abbot Axsym System HCV 3.0), HCV–RNA düzeyleri, 2009-2011 yılları arasında Artus HCV RG PCR kiti (QIAGEN Almanya) kullanılarak, Rotor-Gene 6000 (Corbett Research, Almanya) cihazında, 2011-2014 yılları arasında ise, Cobas TaqMan 48 (Roche, ABD) sistemi kullanılarak Real Time PCR yöntemiyle belirlenmiştir. HCV RNA pozitif hastalarda genotip tayini, 2009-2011 yılları arasında HCV genotip Pyrosequencing testi (QIAGEN), 2011-2014 arası ise Abbott Real Time Genotype II ile (ABD) yapılmıştır. Bulgular: HCV genotip dağılımı: 151 hastada (%74.4) genotip 1; 3 hastada genotip 2 (%1.4), 4 hastada genotip 3 (%1.9), 4 hastada genotip 4 (%1.9) 36 hastada genotip 1b (%17.7), 5'inde genotip 1a (%2.4) olarak saptandı. Hastaların HCV RNA düzeylerinin $1.25x10^3$-$8.35x10^7$ aralığında olduğu belirlenmiştir. Tartışma: Bu araştırmada Eskişehir bölgesinde takip ve tedavi edilen kronik hepatit C hastalarında en sık rastlanan HCV genotipinin, genotip1 olduğu, alttip olarak da genotip 1b olduğu görülmüştür. Bu hastalarda kalıcı viral yanıtın zayıf olabileceği göz önüne alınarak, tedavi protokollerinin buna göre tekrar değerlendirilmesi yerinde bir yaklaşım olacaktır. Bazı HCV genotipleri tüm dünyada yaygın olarak bulunurken, bazı tipler belirli coğrafi bölgelerde daha sıktır. Türkiyede HCV enfeksiyonlarının yaklaşık %90'ı tip 1 (büyük çoğunluğu tip 1b) olup, tip 2, 3 ve 4 HCV enfeksiyonlarına da rastlanmaktadır.

Anahtar Kelimeler

Eskişehir; HCV Genotiplendirme; Kronik Hepatit C Enfeksiyonu; RT-PCR.

Abstract

Aim: Chronic Hepatitis C is a serious disease than can result in long-term health problems. It is known that different genotypes in HCV infections account for differences in disease courses and treatment responses. Our study aimed to determine the HCV genotype distribution of patients with chronic hepatitis C infection. Material and Method: This study investigated the anti-HCV, HCV RNA viral loads, and HCV genotypes of 203 patients who were followedup in Eskisehir Osmangazi University Medical Faculty from2009-2014. Anti-HCV was tested by microparticle ELISA (Abbott AxSYM System HCV 3.0). HCVRNA viral loads were determined by Artus HCV RG PCR kit (QIAGEN) on a Rotor-Gene 6000 (Corbett Research) instrument between 2009-2011, and by Cobas TaqMan 48 (Roche) between 2011-2014 by Real Time PCR. Genotyping of HCV RNA positive patients was performed by HCV genotype Pyrosequencing test and PCR based assay Abbott Real Time Genotype II (USA). Results: The distribution of HCV genotypes was as follows: In 151 (74.4%) patients genotype 1; in 36 genotype 1b (17.7%), in 5 type 1a (2.4%), in 3 genotype 2(1.4%); in 4 genotype 3(1.9%); and in 4 genotype 4(1.9%). HCV viral loads were between $1.25x10^3$-$8.35x10^7$. In 191 (94.0%) patients, anti-HCV was positive and in 12 (6.0%), anti-HCV was negative. Discussion: The most common HCV genotype in the Eskisehir region was genotype 1, and the most common subtype in this group was genotype 1b. Treatment protocols should be reevaluated by taking into consideration that sustained viral response in these patients might be weak. In Turkey, approximately 90% of HCV infections are of type 1 (most are type 1b), although types 2, 3, and 4 HCV infections are also seen.

Keywords

Eskisehir; HCV Genotyping; Chronic Hepatitis C Infection; RT-PCR

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Introduction

Hepatitis C virus (HCV) is an enveloped, positive sense single-stranded RNA virus that belongs to the Flaviviridae family [1]. HCV is most often transmitted in aparenteral way, such as through using intravenous drugs or contaminated blood products and transfusion of blood[2]. There are over 180 million people in the world infected with HCV and it causes approximately 350, 000 deaths every year[2, 3]. HCV is a hepatotropic viral agent and one of the most frequent causes of chronic liver disease; it can cause cirrhosis and hepatocellular carcinoma[1,2]. HCV RNA levels and genotypes play an important role in the prognosis and treatment of chronic hepatitis[1]. HCV represents high genetic heterogeneity. This virus is classified into six major genotypes and more than 100 subtypesas shown by sequence analysis; recently a seventh genotype has been identified[3,4].It is known that genotypes are important predictors for epidemiology, clinical courses,and responses to antiviral therapy. Determination of HCV genotype is essential; it is incorporated into the treatment guidelines for HCV infections, including the 2009 American Association for the Study of Liver Diseases guidelines and the 2011 European Association for the Study of the Liver clinical practice guidelines[5]. The present study investigated the distribution pattern of HCV genotypes in patients with chronic hepatitis C infection in the Eskisehir, Turkey region.

Material and Method

In this study, we retrospectively investigated anti-HCV, HCV RNA viral loads, and HCV genotypes of 203 patients from various clinics followed up in ourfaculty from 2009-2014.

Initially, all the serum samples were tested for anti-HCV by microparticle ELISA assayon an automated system (Abbott AxSYM System HCV 3.0, USA).

HCV RNA viral loads were determined by Artus HCV RG PCR kit (Qiagen, Germany) on a Rotor-Gene 6000 device (Corbett Research, USA) instrument after extraction by Biorobot M48 system (QIAGEN) between 2009-2011, and by Cobas TaqMan 48 (Roche,USA) system after extraction by Cobas t v2.0 kit (Roche, USA) between 2011-2014 by Real Time PCR.

Genotyping of HCV RNA positive patients was performed using the HCV genotype Pyrosequencing technique by PyroMark Q24 (QIAGEN, Germany) between 2009-2011. Two PCR reactions and four pyrosequencing reactions were performed for each sample by amplifying 2 different regions (5'UTR and core) of the HCV genome using one-step RT-PCR kit (Qiagen). After RNA isolation from the samples these two regions were amplified by PCR and two products were obtained. PCR products were immobilized onto Streptavidin-Sepharose (GE) beads. HCV genotypes were determined by pyrosequencing assay using HCV genotype sequencing primers (PyroMark Q24, Qiagen, Hamburg, Germany). Four sequencing primers were used and each sequencing primer was added to the immobilized PCR products. For pyrosequencing, the single-stranded PCR amplicon was hybridized with sequencing primers and the reaction started by addition of one of the nucleotides as standard pyrosequencing. Analysis of sequences was performed using the Pyromark Q24 software (Qiagen).

For genotyping of HCV between 2011-2014,Abbott Real Time Genotype II (m2000,IL,USA) was used. It is a PCR based assay targeting and direct sequencing of 5' untranslated regions (UTR) of the gene for genotypes 1-6 and NS5b for subtypes of 1a/1b.Genotype specific fluorescence labeled oligonucleotide probes were used according to the manufacturer's protocols. The limit of detection is 500 IU/ml for Abbott RT HCV Genotype II.

Results

Eighty-seven (42.86%) of 203 patients were male and 116 (57.14%) were female. The average age of the patients was 54.97 and the age range was 14-77. The distribution of HCV genotypes was as follows: In 151 (74.4%) patients genotype 1; in 36 genotype 1b (17.7%); in 5 type 1a (2.4%); in 3 genotype 2 (1.4%); in 4 genotype 3 (1.9%); in 4 genotype 4 (1.9%) (Table 1). HCV viral loads were between $1.25x10^3$-$8.35x10^7$. In 191 (94.0%) patients anti-HCV was positive and in 12 (6.0%) anti-HCV was negative. Mean HCV RNA level was detected as $1.82x10^6$IU/ml. In one of the patients who was negative for anti-HCV, the HCV RNA level was measured as $4,56x10^5$IU/ml.

The mean HCV RNA level was detectedas $1.92x10^6$ IU/ml(range between 33.8- $2.1x10^7$) in genotype 1. In patients infected with other genotypes, viral load was detected as $6.43x10^5$ IU/ml (range between $1.5x10^4$-$3.3x10^6$).

Table 1. HCV genotype distribution

Type	Count	%
Type 1	151	74.4
Type 1a	5	2.4
Type 1b	36	17.7
Type 2	3	1.5
Type 3	4	2
Type 4	4	2
TOTAL	203	100

Discussion

HCV is an important public health problem because of higher chronicity rates and transmission routes. Diagnosis of HCV infection relies on detecting antibodies to HCV by ELISA [6]. After the ELISA assay for positive patients, viral core antigen (HCV core ag) or viral genomic RNA (HCV RNA) can be used to confirm the diagnosis[7].

In 80-90% of patients, anti-HCV antibodies become positive between six to twelve weeks following exposure. Chronic infection is characterized with high transaminase levels, positive RIBA, and detectable viral RNA load for at least 6 weeks [6]. The patient's age, duration of the disease, alcohol use, histological architecture of the liver, co-infection with other hepatitis viruses, viral load, viral transmission route, replicability of the virus, and RNA transcription errors during replication play important roles in chronic infection development.The clinical course and virological response in patients with chronic hepatitis C are related to the HCV genotype. Interferon-based HCV treatment efficiency is influenced by factors including HCV genotype, initial viral load, and virological response during treatment [8,9].

Several methods have been developed for HCV genotyping such as direct sequencing, restriction fragment length polymor-

phism, DNA hybridization, and multiplex real-time reverse transcription polymerase chain reaction (M-Real-Time RT-PCR)[5,6]. The hepatitis Cvirus has high mutation potentialand it displays phenotypically variability in the short term. As a result, HCV can evade host defense mechanisms, change virulence potential and gain resistance to antiviral agents. Genotypes and mutations are important parameters of response to interferon therapy and clinical outcome in chronic hepatitis C patients [2,6].

HCV genotype and subtype prevalence varies with geographical distribution. According to a recently published study, globally genotype 1 is the most common (46%),followed by genotype 3, genotype 2, andgenotype 4. In Europe and Asia, genotype 1b is commonly seen. Genotype 3 is predominant in Asia and Australia. Genotype 4 is more prevalent in the Middle East. Genotypes 5 and 6 are largely reported from South Africa and Southeast Asia [10,11].

In recent studies about HCV genotyping reported from Turkey [9,12-20], genotype 1b is the most common subtype in our country, as it is in Europe, with a prevalence between 60-100% (Table 2). Similarly, in the present study, the most common HCV genotype in chronic hepatitis C patients followed up in the Eskisehir region was genotype 1 (94.5%), and the most common subtype in this group was genotype 1b (17.75%).Subtype determination could be performed for 41 patients; in 36 of those 41 patients it was genotype 1b.Several studies have reported different HCV genotype determination failure rates (0-44%). Gokahmetoglu et al. [21] have reported thatin 27.3% of cases, subtypes could not be determined with pyrosequencing assay. Determination failure can arise from the handling of the sample, assays sensitivity, or technical procedures.

As seen in table 2, genotype 1a is the second most common type of HCV in most of the studies performed in Turkey. Some of the studies revealed different results; Saglik et al. [22]reported the genotype 3 prevalence as 11.1% in their study, while for Kayseri, Gokahmetoglu et al. [21] reported genotype 4 as 35.6% and Kayman et al. [23]reported genotype 4 as 32%. Borcak et al. [24] found genotype 2 prevalence as 14.5% in Nevsehir. They explained that the different results were due to the fact that these cities have higher tourism rates. Cases with different genotypes may originate from tourists from foreign countries. It is established that social events like migration due to war and touristic journeys affect the epidemiology of infections [11].

All of the HCV genotypes present different infectivity and pathogenicity. For example, Genotypes 1 and 4 are more resistant against interferon therapy than genotypes 2 and 3.Genotypes 2 and 3have 80% sustained response to antiviral therapy, whereas in genotypes 1 and 4 this ratio is approximately 50%; for genotypes 5 and 6 sustained response is between 50% and 80% [6]. Genotype 1b is also associated with poor prognosis and is more resistant than the other genotypes. Especially in patients with genotypes 1 and 4, treatment protocolsshouldbe reevaluated given that sustained viral response in these patients might be weak [5]. Currently the Ministry of Health in Turkey requires that HCV genotypes be systematically determined beforestarting an HCV treatment regimen, since the determination of HCV genotypes is an important treatment factor. Also, monitoring the ribavirin dose, determining the duration of treatment, and virological monitoring proceduresshould be factors intreating chronic HCV infection.

In conclusion, determination of HCV genotypes plays an important roleinchoosing antiviral treatment regimen, duration of treatment, and monitoring of response.Therapeutic regimens of HCV includePegylated interferon (PEG-INF) and ribavirin combinations.HCV genotyping is important not only because of the varying rates of treatment response but also because of the prevalence of different genotypes in Turkey. Therefore, it is important to perform studies about the molecular epidemiologic data of HCV infections and to further determinate the genotype profile in Turkey.

Competing interests

The authors declare that they have no competing interests.

Table 2. List of Hepatitis C genotype studies in Turkey since 2000

Author	Region	Year/N	Assay	Genotypes (%)						
				1	1a	1b	2	3	4	mix
Yarkın et al [12]	Adana, Mersin	2000/72	GS-PCR, AGE	-	14.5	82.2	3.3	-	-	-
Ozacar et al [13]	İzmir	2001/170	Inno-LIPA	-	10	81.2	2.4	0.6	1.2	4.7
Erensoy et al[l4].	İzmir	2002/50	GS-SA	-	30	60	-	-	-	-
Bozdayı et al[15].	Ankara	2004/365	PCR-RFLP	-	11	84	3	1	1	-
Ural et al[9].	Konya	2007/80	SA	-	-	100	-	-	-	-
Çil et al[16].	Diyarbakır	2007/22	SA	-	22.7	72.7		4.5	-	-
Altuglu et al[17].	İzmir	2008/345	RFLP-SA	-	9.9	87.2	0.9	1.4	0.6	-
Ciftci et al [8].	Afyon	2009/34	Multiplex-real time PCR	91.2	-	-	-	-	8.8	-
Özbek et al [18].	Diyarbakir	2009/74	Inno-LIPA	4.1	-	87.8	2.7	5.4	-	-
Kalaycı et al [19].	Afyon	2010/30	Multiplex-real time PCR	3.3	20	63.3	-	-	13.3	-
Gokahmetoglu et al [21].	Kayseri	2011/146	Pyrosequencing	61.7					35.6	
Kayman et al [23].	Kayseri	2012/375	Real-time PCR	2.4	2.4	57.6	3.2	1.1	32	1.3
Saglik et al [22].	Antalya	2014/422	LIPA	5.4	14.7	63.3	3.5	11.1	1.6	-
Borcak et al [24].	Nevsehir	2015/170	GS-PCR	45.1	-	37	14.5	1.2	0.6	-
Uysal et al[20].	Sivas	2016/284	Pyrosequencing	-	6.7	89.4	1.4	1.1	1.4	--
This study	Eskisehir	2016/203	Pyrosequencing-Real-tıme PCR	74.4	2.4	17.7	1.4	1.9	1.9	-

GS-PCR:Genotype spesific-polymerase chain reaction, RFLP: restriction fragment length polymorphism, SA:Sequence analysing, LIPA: Line prob assay

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